AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 1, line 3, with the following rewritten paragraph:

The research in this patent application was supported in part by National Institutes of Health grants CA ~~26380~~ 47956 and CA 60381. The United States government has certain rights in the invention.

Please replace the paragraph beginning on page 3, line 17, with the following rewritten paragraph:

In accordance with preferred embodiments, the anticode oligomer is substantially complementary to a strategic site in the pre-mRNA sense strand or substantially complementary to the mRNA. A preferred strategic site is the translation-initiation site of the pre-mRNA coding strand. Alternative strategic sites include coding sites for splicing, transport or degradation. The subject anticode oligomer either in its "native," unmodified form--oligonucleotide--or as a derivative, is brought into contact with the target lymphoma or leukemia cells. For *in vivo* therapeutic use, a derivative of the "native" oligonucleotide, such as the phosphorothioate form is preferable since it is believed that these forms are more resistant to degradation, notwithstanding the fact that response times to some analogues, such as the phosphorothioate analogs, has been found to be somewhat slower than to the "native" form of the oligo~~y~~nucleotide.

Please replace the paragraph beginning on page 6, line 22, with the following rewritten paragraph:

Figure 2 shows graphs of the concentration dependence of inhibition of cell proliferation by antisense normal and phosphorothioate oligodeoxynucleotides. Oligodeoxynucleotide additions to cultures included TI-AS phosphorothioate (○ and ●; two separate experiments), TI-S phosphorothioate (▲), TI-AS normal (□), and TI-S normal (Δ).

Please replace the paragraph beginning on page 7, line 17, with the following rewritten paragraph:

Figures 8 (a) - (c) show bcl-2 antisense oligodeoxynucleotides producing sequence-specific reductions in bcl-2 mRNA and bcl-2 protein and producing increased sensitivity of SU-DHL-4 cells to cancer chemotherapeutic drugs.

Please replace the paragraph beginning on page 7, line 25, with the following rewritten paragraph:

Figures 10 (a) and (b) ~~(a-b)~~ shows reduction of chemoresistance of RS11846 cells from inducible bcl-2 antisense expression from an expression plasmid.

Please replace the paragraph beginning on page 8, line 21, with the following rewritten paragraph:

The anticode oligonucleotides and analogs thereof may be RNA or DNA, or analogs of RNA or DNA, commonly referred to as antisense oligomers or antisense oligonucleotides. Such RNA or DNA analogs comprise but are not limited to 2-'~~O~~O'-alkyl sugar modifications, methylphosphonate, phosphorothiate, phosphorodithioate, formacetal, 3'-thioformacetal, sulfone, sulfamate, and nitroxide backbone modifications, and analogs wherein the base moieties have been modified. In addition, analogs of oligomers may be polymers in which the sugar moiety has been modified or replaced by another suitable moiety, resulting in polymers which include, but are not limited to, morpholino analogs and peptide nucleic acid (PNA) analogs (Egholm, et al. Peptide Nucleic Acids (PNA)--Oligonucleotide Analogues with an Achiral Peptide Backbone, (1992)).

Please replace the paragraph beginning on page 9, line 4, with the following rewritten paragraph:

Anticode analogs may also be mixtures of any of the oligonucleotide analog types together or in combination with native DNA or RNA. At the same time, the oligonucleotides and

analogs thereof may be used alone or in combination with one or more additional oligonucleotides or analogs thereof. The oligonucleotides may be from about 10 to about 1,000 nucleotides long. Although oligonucleotides of 10 to 100 nucleotides are useful in the invention, preferred oligonucleotides range from about 15 to about 24 bases in length.

Please replace the paragraph beginning on page 10, line 21, with the following rewritten paragraph:

A human gene termed bcl-2 (B cell lymphoma/leukemia-2) is implicated in the etiology of some common lymphoid tumors, Croce et al., "Molecular Basis Of Human B and T Cell Neoplasia," in: *Advance in Viral Oncology*, 7:35-51, G. Klein (ed.), New York: Raven Press, 1987. High levels of expression of the human bcl-2 gene have been found in all lymphomas with t (14; 18) chromosomal translocations including most follicular B cell lymphomas and many large cell non-Hodgkin's lymphomas. High levels of expression of the bcl-2 gene have also been found in certain leukemias that do not have a t(14; 18) chromosomal translocation, including most cases of chronic lymphocytic leukemia acute, many lymphocytic leukemias of the pre-B cell type, neuroblastomas, nasophryngeal carcinomas, and many adenocarcinomas of the prostate, breast, and colon. (Reed et al., Differential expression of bcl-2 protooncogene in neuroblastoma and other human tumor cell lines of neural origin. Cancer Res. 51:6529 (1991); Yunis et al. Bcl-2 and other genomic alterations in the prognosis of large-cell lymphomas. New England J. Med. 320:1047; Campos et al. High expression of bcl-2 protein in acute myeloid leukemia is associated with poor response to chemotherapy. Blood 81:3091-3096 (1993); McDonnell et al. Expression of the protooncogene bcl-2 and its association with emergence of androgen-independent prostate cancer. Cancer Res. 52:6940-6944 (1992); Lu, Q-L, et al. Bcl-2 protooncogene expression in Epstein Barr Virus-Associated Nasopharyngeal Carcinoma, Int. J. Cancer 53:29-35 (1993); Bonner et al. bcl-2 protooncogene and the gastrointestinal mucosal epithelial tumor progression model as related to proposed morphologic and molecular sequences, Lab. Invest. 68:43A (1993)).

Please replace the paragraph beginning on page 12, line 6, with the following rewritten paragraph:

Blocking translation at such strategic sites prevents formation of a functional bcl-2 gene product. It should be appreciated, however, that any combination or subcombination of anticode oligomers, including oliegonucleotides complementary or substantially complementary to the bcl-2 pre-mRNA or mRNA that inhibit cell proliferation, is suitable for use in the invention. For example, oligodeoxynucleotides complementary to sequence portions of contiguous or non-contiguous stretches of the bcl-2 RNA may inhibit cell proliferation and would thus be suitable for use in the invention.

Please replace the paragraph beginning on page 12, line 30, with the following rewritten paragraph:

Preferred antisense, or complementary, oligodeoxynucleotides are listed in Table ~~I~~ I.

Please replace the paragraph beginning on page 13, line 19, with the following rewritten paragraph:

It is preferable to use chemically modified derivatives or analogs of anticode oligomers in the performance of the invention rather than "native" or unmodified oligodeoxynucleotides. "Native" oligodeoxynucleotides can be conveniently synthesized with a DNA synthesizer using standard phosphoramidite chemistry. Suitable derivatives, and methods for preparing the derivatives, include phosphorothioate, Stein et al., Nucl. Acids Res., 16:3209-3221 (1988); methylphosphonate, Blake et al., Biochemistry 24:6132-6138 (1985) and alphadeoxynucleotides, Morvan et al., Nucl. Acids Res.~~,~~ 14:5019-5032 (1986), 2'-O-methyl-ribonucleosides (Monia et al. Evaluation of 2'-modified oligonucleotides containing 2' deoxy gaps as antisense inhibitors of gene expresssion. J. Biol. Chem. 268:14514-14522 (1933)), and covalently-linked derivatives such as acridine, Asseline et al., Proc. Natl Acad. Sci. ~~USA~~ *USA* 81:3297-3201 (1984); alkylated (e.g., N-2-chlorocethylamine), Knorre et al., Biochemie 67:783-789 (1985) and Vlassov et al., Nucl. Acids Res. 14:4065-4076 (1986); phenazine, Knorre et al., supra, and Vlassov et al., supra; 5-methyl-N^4 - N^4-ethanocytosine, Webb et al., Nucl. Acids Res. 14:7661-7674 (1986); Fe-ethylenediamine tetraacetic acid (EDTA) and analogues, Boutorin et al., FEBS Letter~~'~~s 172:43-46 (1984); 5-

glycylamido-1,-10-~~o~~O-phenanthroline, Chi-Hong et al., Proc. Natl. Acad. Sci. ~~USA~~ *USA* 83:7147-7151 (1986); and diethylenetriaamine-pentaacetic acid (DTPA) derivatives, Chu et al., Proc. Natl. Acad. Sci. *USA* 82:963-967 (1985). All of the above publications are hereby specifically incorporated by reference as if fully set forth herein.

Please replace the paragraph beginning on page 14, line 16, with the following rewritten paragraph:

The anticode oligomer of the present invention can also be combined with a pharmaceutically acceptable carrier for administration to a subject or for ex-vivo administration. Examples of suitable pharmaceutical carriers are a variety of cationic lipids, including, but not limited to N-(1-(2,3-dioleyloxy)propyl)-~~n,n,n~~N,N,N-trimethylammonium chloride (DOTMA) and dioleoylphosph~~a~~~~o~~tidylethanolamine (DOPE)]. Liposomes are also suitable carriers for the anticode oligomers of the invention.

Please replace the paragraph beginning on page 15, line 17, with the following rewritten paragraph:

The anticode oligomers may also be useful for *ex vivo* bone marrow purging. Normally, the amounts of conventional cancer chemotherapeutic agents or drugs and irradiation that a patient can receive are limited by toxicity to the marrow, i.e., anemia (fatigue, heart failure), thrombocytopenia (bleeding), neutropenia (infection). Thus, in order to deliver sufficient concentrations of drugs and irradiation to totally eradicate the tumor, the physician would simultaneously destroy the patient's normal bone marrow cells leading to patient demise. Alternatively, large amounts of bone marrow can be surgically extracted from the patient and stored *in vitro*, while the patient receives aggressive conventional treatment. The patient can then be rescued by reinfusion of their own bone marrow cells, but only if that marrow has been "purged" of residual malignant cells. The claimed anticode oligomers could be used to remove residual malignant cells from the bone marrow.

Please replace the paragraph beginning on page 16, line 9, with the following rewritten paragraph:

The anticode oligomers are administered to the patient for at least a time sufficient to inhibit proliferation of the cancer cells. The anticode oligomers are preferably administered to patients at a frequency sufficient to maintain the level of anticode oligomers at an effective level in or around the cancer cells. To maintain an effective level, it may be necessary to administer the anticode oligomers several times a day, daily or at less frequent intervals. Anticode oligomers are administered until cancer cells can no longer be detected, or have been reduced in number such that further treatment provides no significant reduction in number, or the cells have been reduced to a number manageable by surgery or other treatments. The length of time that the anticode oligomers are administered will depend on factors such as the rate of uptake of the particular oligodeoxynucleotide by cancer cells and time needed for the cells to respond to the oligodeoxynucleotide. *In vitro*, maximal inhibition of neoplastic cell growth by "native," unmodified anticode oligomers occurred two days after initiation of cultures,; whereas phosphorothioate oligodeoxynucleotides required 4 to 7 days to achieve maximal inhibition. *In vivo*, the time necessary for maximal inhibition of cell proliferation may be shorter or longer.

Please replace the paragraph beginning on page 17, line 7, with the following rewritten paragraph:

It is also believed that the anticode oligomers of the invention may be useful in the treatment of autoimmune diseases. Autoimmune diseases are those diseases in which the body's immune system has malfunctioned in some way. Administration of the anticode oligomers of the invention to a person having an autoimmune disease should inhibit proliferation of bcl-2 overexpressing lymphocytes, which would in turn reduce the symptoms of the autoimmune disease. For use in treating autoimmune diseases,- the anticode oligomers would be administered as described herein.

Please replace the paragraph beginning on page 18, line 10, with the following rewritten paragraph:

B. Measurement of Cellular Growth. Growth of cell lines cultured in the presence or absence of anticode oligomers was measured by two methods: cell counts using a hemocytometer; and DNA synthesis by assaying [^{3}H]-thymidine incorporation essentially as described in Reed et al., *J. Immunol.*, 134:314-319 (1985). Briefly, cells were cultured in 96-well flat-bottomed microtiter plates (Falcon) at 0.2 ml/well. At appropriate times, cells were resuspended, 25 µl removed from cultures for cell counting, and this volume replaced with 25 µl of 20 UCi/~~ML~~ml [^{3}H]-thymidine (specific activity 6.7 Ci/mmole) (New England Nuclear). Microtiter cultures were then returned to 37° C. and 95% air: 5% C~~0~~O$_2$ atmosphere for 8 hours before lysing cells an glass filters and determining relative levels of [^{3}H]-thymidine incorporation into DNA by scintillation counting. Cell counts were performed in the presence of trypan blue dye to determine the concentration of viable cells in duplicate microcultures.

Please replace the paragraph beginning on page 18, line 29, with the following rewritten paragraph:

MTT [3-(4,–5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] dye reduction assays were performed by the method of Tada, et al. J. Immunol. Methods 93, 157, (1986), and confirmed to be within the linear range of the assay under the conditions described here. The number of viable cells per well was extrapolated from standard curves that were included with each assay and that consisted of serial two-fold dilutions of exponentially growing SU-DHL-4 cells in HL-1 medium, beginning with 10^6 cells/ml (0.2 ml~~mi~~/well). Samples were assayed in triplicate and the $OD600_{nm}$ for a media/reagent blank was subtracted from all values prior to calculations.

Please replace the paragraph beginning on page 20, line 10, with the following rewritten paragraph:

Table ~~I~~ shows the oligodeoxynucleotides synthesized and their relation to the sense-strand of the human bcl-2 gene. Portions of the sequence of the coding strand of the human bcl-2 gene are shown, including the translation initiation site (top), splice donor site (middle), splice acceptor region (bottom), and emperically selected sites within the 5' untranslated portion of bcl-2 pre-mRNA. The ATG initiation codon, GT splice donor, and AG splice acceptor consensus sequences are in boxes.

Please replace the paragraph beginning on page 21, line 1, with the following rewritten paragraph:

In an effort, to specifically block splicing of bcl-2 mRNAs, a 20 bp antisense oligodeoxynucleotide, SD-AS, was synthesized that overlaps the splice donor site in bcl-2 primary transcripts. In addition, a complementary sense oligodeoxynucleotide, SD-S, was prepared as depicted in Table ~~I~~. The human bcl-2 gene gives rise to several transcripts through alternative splice site selections, see Tsujimoto et al., *Proc. Natl. Acad. Sci. USA*, 83:5214-5218 (1986). The preponderance of these transcripts depend upon splicing and encode a 26 kDa protein, bcl-2-alpha. One minor transcript, however, does not undergo a splice and consequently encodes a 22 kDa protein bcl-2-beta. The SD-AS oligodeoxynucleotide can thus potentially block maturation of most but not all bcl-2 transcripts.

Please replace the paragraph beginning on page 22, line 3, with the following rewritten paragraph:

The TI-AS normal oligodeoxynucleotide was more effective in 68~~°~~ C-treated serum at suppressing the growth in culture of these lymphoma cells. In all subsequent experiments, sera heated at 68° C for 1 hour prior to use were used in cultures. This treatment did not impair the growth-supporting capacity of the sera.

Please replace the paragraph beginning on page 27, line 4, with the following rewritten paragraph:

To avoid experimental variation due to differences among lots of sera, 697 leukemic cells were cultured in serum-free conditions. When cultured at an initial seeding density of $0.5x1\cancel{0}\underline{0}^6$ cells/ml, 697 cells achieved maximal DNA synthesis and cellular densities at 4-5 days. Addition of 25 μM sense phosphorothioate oligodeoxynucleotide (TI-S) at the initiation of these cultures had little effect on 697 cell growth. In replicate cultures containing 25 μM antisense phosphorothioate (TI-AS), however, some diminution in DNA synthesis was evident within 2 days and was maximal at 4-5 days. Maximal inhibition of 697 cell growth, as determined by cell counts, was seen at 6 days after initiation of cultures.

Please replace the paragraph beginning on page 27, line 18, with the following rewritten paragraph:

When 697 cells were initially seeded at $0.2x1\cancel{0}\underline{0}^6$ cells/ml, the antisense phosphorothioate oligodeoxynucleotide, TI-AS, resulted in only slight inhibition at 2 days, attaining maximal suppression of DNA synthesis in these cultures at day 7. As with normal oligodeoxynucleotides, this inhibition by phosphorothioate oligodeoxynucleotides appeared to be mediated through non-cytotoxic mechanisms, since cellular viabilities did not decline until late in the course of culture. Compared with normal antisense oligodeoxynucleotides, therefore, phosphorothioate oligodeoxynucleotides had a slower onset of action.

Please replace the paragraph beginning on page 31, line 8, with the following rewritten paragraph:

For RNA blot analyses, polyadenylated mRNA was purified from normal NIH 3T3 cells and from cells stably transfected with expression constructs that produce either sense (3T3-alpha-S) or antisense (3T3-alpha-AS) recombinant bcl-2-alpha mRNAs, according to the method of 13. Approximately 5 μg of mRNA was subjected to RNA blot analysis, essentially as described in (16), using ~~either~~ $^{3}{}_{2P}{}^{32}\underline{P}$-labeled hybridization probes derived from <u>either</u> human or murine bcl-2 sequences.

Please replace the paragraph beginning on page 32, line 26, with the following rewritten paragraph:

Adding TI-S oligodeoxynucleotide to cultures of 3T3-alpha-AS cells (produce antisense bcl-2 transcripts) ruled out inhibition of cellular growth through a nonspecific mechanism involving oligodeoxynucleotide-RNA hybrid formation. The TI-S oligodeoxynucleotide caused little suppression of 3T3-alpha-AS cell proliferation, whereas the TI-AS oligodeoxynucleotide was markedly inhibitory in these cells. Similar data were obtained with TI-AS and TI-S phosphorothioate oligodeoxynucleotides.

Please replace the paragraph beginning on page 33, line 3, with the following rewritten paragraph:

Example 12

Measurements of DNA Fragmentation as an Indicator of bcl-2 Antisense Oligodeoxynucleotide-Mediated Programmed Cell Death in Human Lymphoma Cells

Oligonucleotides having the sequences shown in Table 2 were tested for the ability to induce programmed cell death (DNA fragmentation) in the human t(14:18)-containing human lymphoma cell line RS11846. The oligonucleotides were all phosphodiesters, and were targeted against the translation initiation site or the 5'-cap region of bcl-2 pre-mRNAs. Control oligodeoxynucleotides included a bcl-2 sense version (TI-S) of TI-AS (having SEQ ID NO: 7) and a scrambled version of T~~I~~I-AS that has the same base composition, but with jumbled nucleotide order.

Please replace the paragraph beginning on page 33, line 28, with the following rewritten paragraph:

RS11846 cells were adapted to grow in HL1 media with 1% FCS and their DNA metabolically labeled by addition of ^{125}I-deoxyuridine to cultures for three hour. Labeled cells were

then washed thoroughly and cultured for two days in the presence or various oligonucleotides at 50 ~~A~~μM. Cells were then recovered from 200 μL cultures by centrifugation, and lysed in a hypotonic buffer containing 10 mM EDTA and 14 Triton X100. After centrifugation at 16,000 x_g to pellet unfragmented genomic DNA, the supernatant fraction containing fragmented DNA was extracted with phenol/chloroform and ethanol precipitated. This DNA was then subjected to gel electrophoresis in 1.5% agarose gel and transferred to nylon membranes for autoradiography.

Please replace the paragraph beginning on page 34, line 21, with the following rewritten paragraph:

The presence of a ladder of DNA fragments (unit size of approximately 200 bp) is indicative of programmed cell death. At 50 μM, TI-AS caused little DNA fragmentation, whereas the oligonu~~e~~cleotides having SEQ ID NO: 9 and SEQ ID NO: 10, and one of the 5'-cap oligonucleotides (SEQ ID NO: 14) led to pronounced DNA fragmentation.

Please replace the paragraph beginning on page 34, line 28, with the following rewritten paragraph:

Example 13

Concentration-Dependence of Inhibition by Antisense Phosphodiester Oligodeoxynucleotides in Serum-Free Cultures

697 pre-B cell leukemia cell were cultured in medium with either 1% (vol:vol) HL-1 concentrate (serum-free conditions [o] or 3%, (vol:vol) 68° C-treated serum (FBS2) [●], see Figure 5. Shown are cellular densities measured after 2 days in cultures containing various concentrations of phosphodiester TI-AS oligodeoxynucleotide. Data are shown as percentages relative to control cultures treated with a sense oligonucleotide, and reflect the mean ± standard deviation for duplicate samples.

Please replace the paragraph beginning on page 37, line 14, with the following rewritten paragraph:

As shown in Figures 6 (a) and (b), PO and PS *bcl-2* antisense oligonucleotides produced specific concentration-dependent reductions in the levels of *bcl-2* proteins, without altering the levels of expression of HLA-DR (Figure 7) and other control antigens. At 150 μM, for example, PO antisense oligodeoxynucleotide caused an approximately 75-95% reduction in *bcl-2* fluorescence, whereas the control sense oligodeoxynucleotide diminished *bcl-2* protein levels by only 10-20% (Figure 6(a)). Similarly, cultured 697 cells for 4 days with the PS antisense oligodeoxynucleotide ar 25 μM resulted in approximately 70% reduction in *bcl-2* fluorescence. In comparison, the sense PS oligodeoxynucleotide TI-AS inhibited *bcl-2* protein levels by only approximately 15%, as measured by this assay (Figure 6(b)).

Please replace the paragraph beginning on page 37, line 30, with the following rewritten paragraph:

In phosphorothioate oligodeoxynucleotides, one of the non-bridging oxygen atoms in each internucleotide phosphate linkage is reseplaced by a sulfur atom. This modification renders phosphorothioate oligodeoxynucleotides extremely resistant to cleavage by nucleases, Stein et al., *Nucl. Acids Res.*, 16:3209-3221 (1988). Despite the substitution of a sulfur atom for an oxygen, phosphorothioate oligodeoxynucleotides retain good solubility in aqueous solutions; hybridize well, though with some decrease in the melting temperature of RNA-oligodeoxynucleotides duplexes; and are synthesized conveniently by the widely employed method of automated oligodeoxynucleotides synthesis with phosphoroamidites.

Please replace the paragraph beginning on page 40, line 31, with the following rewritten paragraph:

MCF-7 is a human breast adenocarcinoma cell line that contains relatively high levels of bcl-2 protein. The cells were cultured at 4,000 cells per well in 96-well microtiter plates in the

presence or absence of MP/PO oligomers. Relative cell numbers per well were then estimated by MTT assay, based on a standard curve prepared using freshly plated, untreated MCF-7 cells. The antisense (As) and scrambled (Sc) MP/PO oligomers were the same as those described in Example 165. Data represent the mean +/- standard deviation for determinations.

Please replace the paragraph beginning on page 41, line 9, with the following rewritten paragraph:

The results demonstrate sequence specific inhibition of growth of solid tumor cells by the ~~the~~ claimed anticode oligomer analogs.

Please replace the paragraph beginning on page 41, line 19, with the following rewritten paragraph:

DoHH2 lymphoma cells were treated with various concentrations of oligomers targeted to different sites on the bcl-2 mRNA. The ATG oligomer (SEQ ID NO:17) targets the translation initiation site, and is complementary to the first 6 codons of the open reading frame. The Dscore 23 and Dscore 72 oligomers (SEQ ID NOS:26 and 28, respectively) target sites in the 5' untranslated region of the mRNA. Sc oligomers (SEQ ID NOS:25, 27 and 29) represent negative controls having the same length and base composition but in scrambled order. All oligomers were prepared as phosphodiester (PO)/phosphorothioate (PS) chimeras, where only the last (3') two internucleoside linkages were phosphorothioates. Oligomers were added directly to cultures and relative numbers of viable cells were estimated by MTT assay 3 days later. Data in Figure 13 represent mean +/- standard deviation.

Please replace the paragraph beginning on page 43, line 1, with the following rewritten paragraph:

Antimetabolites include, but are not limited to, methotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, hydroxyurea, 2~~0~~-chlorodeoxy adenosine.

Please replace the paragraph beginning on page 43, line 5, with the following rewritten paragraph:

Alkylating agents include, but are not limited to, cyclophospham~~;~~ide, melphalan, busulfan, cisplatin, paraplatin, chlorambucil, and nitrogen mustards.

Please replace the paragraph beginning on page 43, line 8, with the following rewritten paragraph:

Plant alkaloids include, but are not limited to, vincristine, vinblastine, and VP-16.

Please replace the paragraph beginning on page 43, line 28, with the following rewritten paragraph:

The methods for preparing and purifiying the antisense (AS) and scrambled (SC) 18~~'~~-mer oligonucleotides used for the present work are described above in General Methods and in Kitada et al. (Antisense R & D, 3:157 (1993)). Phosphodiester oligonucleotides were synthesized in a 10-15 micromole scale using phosphoroamidate chemistry with oxidation by iodine, and then purified using a C_{18}-reverse phase column. In most cases, oligomers were additionally ethanol-precipitated five times to eliminate any nonspecific cytotoxic activity, and then dried and resuspended in sterile HL-1 medium (Ventrex Labs, Inc; Burlingame, Calif.) at 1-10 mM. The pH of this solution was adjusted using 1-10 M NaOH until the phenol red indicator dye in the media returned to its original color.

Please replace the paragraph beginning on page 46, line 11, with the following rewritten paragraph:

Aliquots of 1.5 ml of HL-1 serum-free medium (Ventrex Labs, Inc.) supplemented with 1 mM L-glutamine, 50 Units/ml penicillin, and 100 ~~u~~μg/ml streptomycin and either 5 ~~u~~μg of purified oligonucleotides or 30 ~~u~~μg of Lipofectin^R. [1:1 w/w mixture of N-(1-(2,3-dioleyloxy)propyl)-~~n,n,n~~N,N,N-trimethylammonium chloride (DOTMA) and

dioleoylphophotidylethanolamine (DOPE)] were combined and added to 0.75 X 10^6 SU-DHL-4 cells in 3 mls of HL-1 medium. Cells were then either cultured at 37° C. in a humidified atmosphere of 5% CO_2 /95% air in 24 well plates (2 mls/well) for immunoblot and RT-PCR assays, or in 96-well flat-bottom microtiter plates (0.1 ml/well) for MTT assays. For cells in microtiter cultures, typically 0.1 ml of additional HL-1 media with or without various chemotherapeutic drugs was added after 1 day, and the cells were cultured for an additional 2 days before performing MTT assays.

Please replace the paragraph beginning on page 46, line 28, with the following rewritten paragraph:

Cells were washed once in PBS, lysed in a buffer containing 1% Triton X100, and samples normalized for protein content (25 ~~u~~μg) prior to size-fractionation of proteins by SDS-PAGE (12% gels) and transfer to nitrocellulose filters for immunoblot assays as described in Reed et al. Cancer Res. 51:6529 (1991). Preliminary experiments determined that aliquots of lysates containing 25 ~~u~~μg of total protein produced results in the linear range of the assay. Blots were first incubated with 0.1% (v.v) of a rabbit antiserum directed against a synthetic peptide corresponding to amino-acids (aa) 41-54 of the human Bcl-2 protein, as shown in SEQ ID NO. 21 (id) followed by 2.8 ~~u~~μg/ml biotinylated goat anti-rabbit IgG (Vector Labs, Inc.). Bands corresponding to p26-Bcl-2 were then visualized by color development using a Horseradish Peroxidase (HRP)-avidin-biotin complex reagent (Vector Labs, Inc) and 3,3'-diaminobenzidine (DAB). Stained blots were then incubated with a second anti-Bcl-2 antibody directed against aa 61-76 of the Bcl-2 protein (SEQ ID NO. 21) followed by 0.25 ~~u~~μCi/ml ^{125}~~I~~I-protein A. Bcl-2 bands were excised from the blots and subjected to gamma-counting.

Please replace the paragraph beginning on page 47, line 25, with the following rewritten paragraph:

Anticode specific reductions in the relative levels of bcl-2 mRNA were detected within 1 day by a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay. See Figure 8A.

Please replace the paragraph beginning on page 48, line 3, with the following rewritten paragraph:

In Figure 8B, SU-DHL-4 cells were cultured with pairs of either PS (squares) or PO/PS (circles) As- and Sc-oligomers for 3 days. Relative levels of Bcl-2 protein were then measured using a quantitative immunoblot assay, as described above, and the data expressed as a percentage relative to cells treated with control Sc-oligomers. The inset shows immunoblot results for p26-Bcl-2 and a p75 8 cross-reactive (CR) band in a case where As-PO/PS oligomer produced a 41% relative decrease in Bcl-2 protein levels. In Figure 8C, 10^{-4}M Ara-C, MTX, or DEX was added 1 day after addition of PS (squares) or PO/PS (circles) oligomers to cultures of SU-DHL-4 cells, and MTT assays were performed on day 3. Data are presented as a % control relative to cells cultured with drugs in the absence of any oligomers, and represent the results of 9 of 10 consecutive experiments [in one experiment, the MTT assay failed]. Similar results were obtained when dye exclusion assays were used to assess cell survival rather than MTT assay [not shown].

Please replace the paragraph beginning on page 50, line 5, with the following rewritten paragraph:

In the present work, preliminary studies demonstrated that more than 90% of SU-DHL-4 cells survived treatment for 4 days with high dose (10^{-4}) Ara-C, MTX or DEX, presumably because of their high levels of bcl-2 protein (Not shown). At these concentrations, however, all drugs induced essentially complete inhibition of SU-DHL-4 cell proliferation, consistent with bcl-2 convertsing drugs from cytotoxic to cytostatic. Comparisons of AS and SC oligomers demonstrated that bcl-2 AS treatment markedly enhanced the sensitivity of these lymphoma cells to MTX and Ara-C, and to a lesser extent to DEX (Figure 8C).

Please replace the paragraph beginning on page 51, line 14, with the following rewritten paragraph:

Treatment of 32D cells with oligomer/cationic lipid complexes was as described above except that 50 Units/ml of murine recombinant IL-3 (r~~I~~IL-3) was included in the HL-1 media, the initial cell density was 10^5 per ml, and replication-defective adenovirus dl312 (MOI=200) was added 30 minutes after exposure of cells to oligomers to facilitate exit of DNA from endosomes [Yoshimura K, et al. J Biol Chem. 268, 2300, (1993)].

Please replace the paragraph beginning on page 52, line 19, with the following rewritten paragraph:

Figure 9 compares the sensitivity of 32D-BCL-2 and 32D-BHRF-1 cells to various concentrations of MTX. Treatment with bcl-2 AS-oligomers resulted in sequence-specific increases in the sensitivity of 32D-BCL-2 cells to inhibition by MTX at concentrations of 10^{-6} to 10^{-4} M ($P \leq 0.001$ for AS versus SC). In contrast, treatment with bcl-2 AS oligomers produced no significant difference in the sensitivity of 32D-BHRF-1 cells to MTX, relative to control SC-oligomers (Figure 9). These data indicate that the effects of bcl-2 AS oligomers on chemosensitivity to cytoxic agents drugs are sequence specific. Furthermore, several other control oligomers, including bcl-2 sense, other scrambled sequences with the same nucleoside composition as AS, and oligomers with totally unrelated sequences all had comparatively little effect on the chemosensitivity of the cells (Not shown).

Please replace the paragraph beginning on page 53, line 13, with the following rewritten paragraph:

A different strategy was employed to determine if AS-mediated reductions in bcl-2 gene expression could be achieved with an inducible bcl-2 AS expression plasmid that used a heavy metal responsive the human metallothionein-IIA promoter in another translocation t(14;18)-containing lymphoma line, RS11846. RS11846 was obtained from Dr. Carlo Croce (Wistar Institute, Philadelphia, PA) (Tsujimoto and Croce, Proc. Natl. Acad. Sci. USA 83:5214 (1986)).

Please replace the paragraph beginning on page 53, line 22, with the following rewritten paragraph:

To prepare the expression plamid, a 0.91 kbp bcl-2 cDNA (ibid)~~)~~ was subcloned in either antisense (AS) or sense (S) orientation~~o~~ into a HindIII site downstream of a human metalothionein-IIA promoter in the plasmid pMEP-4 (Invitrogen, Inc.), which contains a hygromycin phosphotransferase gene and the EBNA-1 gene and origin of DNA replication from Epstein-~~-V~~Barr Virus for high copy episomal maintenance.

Please replace the paragraph beginning on page 53, line 30, with the following rewritten paragraph:

RS11846 cells (5 X 10^6) in Dulbecco's phosphate buffered saline containing 30 ug of plasmid DNA were electroporated (1500 uF, 270 V/cm) using a Cellject Electroporation System from EquiBio, Inc. Cells were returned to their usual culture media (RPMI-L 1640 supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 50 Units/ml penicillin, and 100 ug/ml streptomycin) at 2 X 10^5 cells per ml and cultured for 2 days before seeding cells at 2 X 1~~0~~0^5 per ml in media containing 200 ~~u~~µg/ml hygromycin. After 3 weeks of culture, the resulting bulk cell lines were passaged in successively higher concentrations of hygromycin in 200 ~~u~~µg/ml increments until the concentration reached 1 mg/ml (about 4 weeks).

Please replace the paragraph beginning on page 54, line 10, with the following rewritten paragraph:

Hygromycin-resistant RS11846 cell were cultured in RPMI/10% serum media containing 0.5 ~~u~~µM $CdCl_2$ and 3 days later immunoblot assays were performed using 25 ug protein/lane essentially as described in Tanaka S, et al. J. Biol. Chem. 268, 10920 (1993) and in Reed et al. Cancer Res. 51:6529 (1991)).

Please replace the paragraph beginning on page 55, line 4, with the following rewritten paragraph:

Preliminary experiments demonstrated that RS11846 cells tolerated the addition of up to 0.5 ~~micro~~μM $CdCl_2$ or to ~~micro~~ 50 μM $ZnCl_2$ to cultures for one week, experiencing a slight decrease in growth rate but essentially no decline in percentage cell viability (Not shown).

Please replace the paragraph beginning on page 55, line 9, with the following rewritten paragraph:

In the absence of heavy metal induction, the relative levels of bcl-2 protein in RS11846 cells containing the control or bcl-2 AS plasmid were comparable, as determined by immunoblot assays (Not shown). When 0.5 ~~u~~μM $CdCl_2$ or 50 ~~u~~μM $ZnCl_2$ was added, reductions in bcl-2 protein became evident in the AS-expressing cells at 2 days and maximal inhibition of 30-40% was obtained at three to four days, relative to control RS11846 cells.

Please replace the paragraph beginning on page 55, line 18, with the following rewritten paragraph:

Figure 10A shows an example of immunoblot lot data derived from RS11846 cells af~~aa~~ter three days of exposure to 0.5 mM $CdCl_2$, demonstrating reduced levels of bcl-2 protein in the AS-plasmid containing cells compared to RS11846 cells that harbored the control plasmid. The relative levels of a control mitochrondrial protein F_1 -beta-ATPase were comparable in all cell lines, consistent with sequence-specific alterations in bcl-2 protein levels.

Please replace the paragraph beginning on page 55, line 27, with the following rewritten paragraph:

When RS11846 cells containing either the control or bcl-2-As plasmids were cultured or various times in 0.5 ~~u~~μM $CdCl_2$, or 50 ~~u~~μM $ZnCl_2$, no significant difference in the growth rates of these two cell lines was observed (Figure 8B). Thus, As-mediated reductions in Bcl-2 protein levels by themselves did not impair RS11846 cell proliferation or survival.

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claims 1-52 (canceled)

Claim 53 (previously presented): An anticode oligomer complementary to bcl-2 mRNA consisting of from 18-35 bases and comprising the nucleotide sequence TCTCCCAGCGTGCGCCAT (SEQ ID NO:17).

Claims 54-69 (canceled)

Claim 70 (currently amended): An anticode oligomer, wherein said anticode oligomer is from 10 to 40 bases in length and is complementary to a portion of ~~SEQ ID NO:19~~ a human bcl-2 mRNA or a portion of a human bcl-2 primary transcript.

Claim 71 (canceled)

Claim 72 (currently amended): The anticode oligomer of Claim 70, wherein said anticode oligomer is an antisense oligonucleotide complementary to a splice acceptor site of a human bcl-2 primary transcript ~~SEQ ID NO:19~~.

Claim 73 (currently amended): The anticode oligomer of Claim 70~~1~~, wherein said anticode oligomer is an antisense oligonucleotide complementary to a splice donor site of a human bcl-2 primary transcript ~~SEQ ID NO:22~~.

Claim 74 (currently amended): The anticode oligomer of Claim 70, wherein said anticode oligomer is 10 to 40 bases in length and is complementary to a 5'-untranslated region of a human bcl-2 mRNA or a human bcl-2 primary transcript ~~SEQ ID NO:19~~.

Claim 75 (currently amended): A composition comprising the anticode oligomer of Claims 53, 70, ~~71,~~ 72, 73 or 74; and a pharmaceutically acceptable carrier.

Claim 76 (previously presented): The anticode oligomer of Claim 53, wherein said anticode oligomer contains at least one phosphorothioate-modified nucleotide.

Claim 77 (previously presented): A composition comprising the anticode oligomer of Claim 76; and a pharmaceutically acceptable carrier.

Claim 78 (previously presented): The anticode oligomer of Claim 76, wherein said anticode oligomer is a phosphodiester/phosphorothioate chimera.

Claim 79 (previously presented): The anticode oligomer of Claim 76 wherein the oligonucleotide comprises at least 2 to 3 phosphorothioate linkages.

Claim 80 (previously presented): A composition comprising the anticode oligomer of Claim 78 or 79; and a pharmaceutically acceptable carrier.

Claim 81 (previously presented): The anticode oligomer of Claim 53, wherein said anticode oligomer contains at least one phosphoramidate-modified nucleotide.

Claim 82 (currently amended): The anticode oligomer of Claims 70, ~~71,~~ 72, 73 or 74, wherein said anticode oligomer contains at least one phosphorothioate-modified nucleotide.

Claim 83 (previously presented): A composition comprising the anticode oligomer of Claim 82; and a pharmaceutically acceptable carrier.

Claim 84 (previously presented): The anticode oligomer of Claim 82, wherein said anticode oligomer is a phosphodiester/phosphorothioate chimera.

Claim 85 (previously presented): The anticode oligomer of Claim 84 wherein the oligonucleotide comprises at least 2 to 3 phosphorothioate linkages.

Claim 86 (previously presented): A composition comprising the anticode oligomer of Claim 84 and a pharmaceutically acceptable carrier.

Claim 87 (previously presented): A composition comprising the anticode oligomer of Claim 85 and a pharmaceutically acceptable carrier.

Claim 88 (currently amended): The anticode oligomer of Claim 70, ~~71,~~ 72, 73 or 74, wherein said anticode oligomer contains at least one phosphoramidate-modified nucleotide.